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Thanks!

Advances in Brief

Serum Bone Sialoprotein in Patients with Primary Breast Cancer Is a Prognostic Marker for Subsequent Bone Metastasis

Ingo J. Diel,[1] Erich-Franz Solomayer, Markus J. Seibel, Johannes Pfeilschifter, Hartwig Maisenbacher, Christina Gollan, Martin Pecherstorfer, Renate Conradi, Gudrun Kehr, Erwin Boehm, Franz P. Armbruster, and Gunther Bastert

Departments of Obstetrics and Gynecology [I. J. D., E. F. S., C. G., H. M., G. K., E. B., F. P. A., G. B.], Endocrinology [M. J. S., J. P.], and Anesthesiology [R. C.], University of Heidelberg, 69115 Heidelberg, Germany, and Department of Medical Oncology, Wilhelminenspital, 1160 Vienna, Austria [M. P.]

Abstract

Bone sialoprotein (BSP) is a noncollagenous bone matrix protein that is important for both mineralization and cell-cell interactions. Tissue studies in primary breast cancers have shown that immunohistochemical expression of BSP is associated with a high incidence of bone metastases in the course of the disease. We used a RIA to investigate the importance of serum BSP as a marker for subsequent bone metastases. Between 1994 and 1996, preoperative blood samples were collected from 388 consecutive patients with nonmetastatic breast cancer and from 30 control patients with benign breast disease. Serum BSP concentrations were measured in a blinded fashion by RIA. The cutoff for elevated serum BSP values was 24 ng/ml, *i.e.*, two SDs above the normal mean value. Serum BSP was correlated with the risk of metastasis and analyzed with regard to its prognostic value. After a median follow-up period of only 20 months, 28 patients had developed metastases. Fourteen patients had bone metastases only, 9 visceral metastases only, and 5 a combination of osseous and visceral metastases. Of the 19 women with skeletal metastases, 17 had preoperative serum BSP values in excess of 24 ng/ml (median BSP values: 48.3 ng/ml for isolated metastatic bone disease, 30.6 ng/ml for combined metastases), whereas none of the women with visceral metastases only had elevated serum BSP concentrations (median BSP value: 12.3 ng/ml). The median serum BSP value in the control group (benign breast disease) was 8.8 ng/ml serum BSP; levels correlated with the size of the primary tumor, but not with any other prognostic factors. Using a multivariate regression analysis, serum BSP was found to be the most important independent prognostic factor for the development of skeletal metastasis ($P < 0.001$; relative risk, 94); its specificity was 96.7%, and its sensitivity was 89.5%. Our study shows that patients with preoperatively elevated serum BSP levels are at high risk of subsequent bone metastases in the first years after primary surgery. The mechanism of BSP in the pathogenesis of skeletal metastases is unclear. Because BSP contains an integrin recognition sequence, its expression in tumor cells may facilitate their adhesion to the bone surface. However, it is possible that a proportion of circulation BSP is derived from normal or tumor-induced bone turnover. Breast cancer patients with elevated serum BSP levels may benefit from osteoprotective adjuvant therapy with bisphosphonates.

Introduction

Bone metastases commonly occur in the course of malignant tumor disease. Although any tumor may metastasize to bone, only five solid tumors (carcinomas of the breast, prostate, lung, kidney, and thyroid) are responsible for >80% of all skeletal metastases. Approximately 75% of women who die of breast cancer display bone metastases at autopsy (1, 2).

For many years, attempts have been made to identify factors for metastatic organ selection in breast cancer to understand the pathogenetic processes of skeletal metastasis. It is known, for example, that well-differentiated and/or steroid receptor-positive breast tumors are more likely to produce skeletal metastases (3–5). Furthermore, there is a correlation between immunohistochemical detection of PTHrP[2] in the primary tumor and osteotropic metastasis (6, 7).

BSP is a glycosylated and phosphorylated protein with a high content of sialic acid (15% of carbohydrates). Like osteocalcin and osteopontin, BSP is one of the noncollagenous proteins in the extracellular bone matrix. It plays an important role in the mineralization and in the adhesion of osteoclasts to the bone surface (8–13). In the last few years, there has been increasing evidence that breast cancers that express BSP immunohistochemically metastasize to bone more frequently than BSP-negative tumors (14, 15).

Recently, a specific RIA has been developed for the measurement of human BSP in serum (16, 17). The aim of our study was to determine serum BSP preoperatively in patients with primary breast cancer and to assess its value as a prognostic factor for subsequent metastatic disease.

Patients and Methods

Patients. The study was carried out in 388 consecutive patients with primary breast cancer who underwent surgery at the Women's Hospital of the University of Heidelberg between October 1994 and October 1996. The inclusion criteria were histologically confirmed breast cancer (T_1–T_4, N_0–N_2, M_0) and the consent of the patient to preoperative blood collection. The exclusion criteria were diagnosis of distant metastases in the perioperative period, previous or simultaneous secondary malignant disease, neoadjuvant chemotherapy or hormone therapy,

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[1] To whom requests for reprints should be addressed, at Department of Obstetrics and Gynecology, University Hospital, Voss-Str. 9, 69115 Heidelberg, Germany.

[2] The abbreviations used are: PTHrP, parathyroid hormone-related protein; BSP, bone sialoprotein; RR, relative risk; UICC, Union International Contre Cancer.

known metabolic bone disease (including clinically apparent osteoporosis with fractures), therapy with substances with a pronounced effect on bone metabolism (exception: calcium supplements and hormone replacement therapy with estrogens/progestins), severe disturbances of liver and kidney function, and pregnancy. To exclude apparent distant metastases, liver ultrasounds. chest X-rays, and bone scans were performed in all patients perioperatively. Computed tomography was carried out in the event of suspicious skeletal findings. Patients with suspected or evident bone disease were excluded from the analysis.

The control group consisted of 30 consecutive patients with histologically confirmed benign breast lesions (21 fibroma/fibroadenoma, 6 cystic mastopathy, and 3 papilloma).

Sample Collection

Immediately before anesthesia and surgery (between 8 and 12 a.m.), 10 ml of blood were collected from all 418 patients via the cubital vein using a syringe without additive. The blood was allowed to clot for 30–45 min at room temperature and then centrifuged immediately (1000 × *g* for 10 min). Aliquots of serum were frozen at −20°C within 2 h of collection.

Determination of BSP in Serum

The serum concentrations of human BSP were determined by an RIA (Immundiagnostik, Bensheim, Germany) using a polyclonal chicken antibody against purified human BSP (16–19). In brief, 100 μl of serum were mixed with 100 μl of chicken antihuman BSP antibody (dilution, 1:200) and 100 μl of ^{125}I-labeled BSP. After incubation for 24 h at 4°C, 100 μl of second-antibody solution (raised in donkeys against chicken IgY; 1:15) were added. After incubating for an additional 2 h at 4°C, the reaction mixture (containing antibody-bound radioactivity) was centrifuged for 10 min at 2000 × *g*. The supernatant was discarded, and the radioactive pellet was washed with 250 μl of an aqueous buffer (60 g/l polyethylene glycol and 9 g/l sodium chloride) before being centrifuged again (10 min at 2000 × *g*). After aspiration of the supernatant, the radioactivity in the pellet was measured in a Berthold gamma counter for 1 min.

The results were calculated by interpolation of the unknown samples with a calibration curve (constructed by use of a four-parameter curve-fitting algorithm). The intraassay coefficient of variation was 5.3% (mean concentration, 9.8 ng/ml; n = 20). The lower limit of detection in the present RIA (determined by 20 replicate analyses of the zero calibrator and calculation of the concentration corresponding to the 95th percentile of the counts obtained) was 0.7 ng/ml. The assay showed a recovery rate of 99.4% (range, 92–108%). All analyses were performed in duplicate within a single run.

The normal serum BSP value is 9.0 ± 3.8 ng/ml in premenopausal women and 13.3 ± 4.8 ng/ml in postmenopausal women (n = 90; age, 20–80 years; Ref. 16). A cutoff for normal BSP values of 24 ng/ml was selected for the statistical analysis of the 418 serum samples from our patients, which corresponds to 2 SDs above the normal value for postmenopausal women.

Therapy of the Patients

The primary surgical therapy consisted of either breast-conserving measures (lumpectomy or segmental resection plus 50 Gy of radiotherapy to the breast) or modified radical mastectomy for large tumors and multicentric/multifocal lesions. Axillary lymph node dissection (levels I and II) was performed in all patients. Tumor tissue was collected from the primary tumor for determination of steroid hormone receptors and S-phase fraction and for tumor grading.

Table 1 Clinical and pathological characteristics of 388 breast cancer patients

Prognostic factor[a]	No. of patients	BSP ≥24		BSP <24	P^b
		n	%	n	
Tumor size					
T_1	195	8	(4)	187	
T_2	156	15	(10)	141	0.001
T_3	25	2	(8)	23	
T_4	12	4	(33)	8	
Nodal status					
N_0	252	16	(6)	236	0.251
N_+	136	13	(9)	123	
Estrogen receptor (n = 357)					
Positive[c]	226	18	(8)	208	0.885
Negative	146	11	(7)	120	
Progesterone receptor (n = 358)					
Positive[c]	228	19	(8)	209	0.831
Negative	130	10	(8)	120	
Menopausal status					
Pre	127	8	(6)	119	0.539
Post	261	21	(8)	240	
Grading (n = 377)					
I + II	247	19	(8)	228	1.000
III	130	10	(8)	120	
S-phase fraction (n = 358)					
<5%	106	11	(10)	95	0.243
≥5%	252	17	(7)	235	
Osteocalcin (n = 264)					
<15 ng/ml	111	7	(6)	104	0.887
≥15 ng/ml	153	9	(1)	144	

[a] Tumor staging according to UICC criteria.
[b] χ^2 test for contingency tables.
[c] Positive, ≥20 fmol/mg protein.

Decisions on adjuvant systemic therapy were based on the guidelines of the St. Gallen Consensus Meetings and the recommendations of the German Adjuvant Breast Cancer Group. All patients with axillary lymph node metastases and those with negative axilla but with other factors suggesting a poor prognosis were given systemic cytotoxic therapy. Serum BSP values were not taken into account when making therapeutic decisions. Patients were treated with 30 mg of tamoxifen daily for 2 years (237 patients); six cycles of standard cyclophosphamide (600 mg/m^2), methotrexate (40 mg/m^2), and 5-fluorouracil (600 mg/m^2; 82 patients); six cycles of cyclophosphamide (600 mg/m^2) and epirubicin (60 mg/m^2) with or without 5-fluorouracil (600 mg/m^2; 32 patients); 3.6 mg of goserelin monthly for 2 years (7 patients), or with a combination of tamoxifen and cyclophosphamide, methotrexate, and 5-fluorouracil (standard CMF regimen; 19 patients). Eleven patients received no adjuvant systemic treatment.

Follow-Up

Follow-up investigations were carried out at the Women's Hospital of the University of Heidelberg. The interval between the investigations was 2–4 months in the first 2 years. Every visit consisted of history and physical examination, whereas chest X-ray, bone scan, liver ultrasound, and mammography were performed yearly. Laboratory tests (blood count, serum tumor antigens, and tumor markers) were carried out at various intervals. If there was evidence of bone metastases, additional X-rays were taken of the areas affected. The pattern of metastasis was analyzed at the end of the study. Bone lesions seen on radiograms were assessed by two independent radiologists.

Table 2 Individual characteristics of patients with metastatic disease

Patients initials	Menopausal status	Tumor size[a]	Nodal status[a]	Estrogen receptor[b]	Progesterone receptor[b]	Tumor grading[a]	S-phase fraction[c]	Localization of first metastases[d]	BSP (ng/ml)
B.A.	Post	2	Pos	765	457	2	7.9	o	34.86
D.E.	Post	2	Pos	248	539	2	3.5	o	54.84
G.P.	Pre	4	Pos	1	1	2	1.9	o	47
H.M.	Post	2	Neg	2	7	3	2	o	96.9
K.U.	Post	1	Neg	12	58	1	5	o	67.25
L.T.	Post	1	Pos	12	78	1	1	o	120
M.H.	Pre	2	Pos	1	1	3	5	o	24.14
M.G.	Pre	1	Neg	23	12	2	3	o	9.53
P.A.	Post	2	Neg	12	55	3	5	o	29.57
R.I.	Post	3	Neg	56	556	1	2	o	44.6
S.K.	Pre	2	Neg	12	13	2	3.7	o	152.9
S.G.	Post	2	Pos	718	75	3	8.8	o	16.98
W.L.	Post	1	Neg	10	1	2	3	o	49.59
W.E.	Post	3	Neg	23	120	3	3.9	o	72.3
B.E.	Post	4	Pos	73	250	2	6.3	v + o	34.8
D.K.	Pre	2	Neg	33	4	2	1.1	v + o	59.5
M.E.	Post	2	Neg	33	11	2	—	v + o	25.48
M.D.	Pre	2	Pos	29	41	3	9.6	v + o	30.59
P.E.	Pre	4	Pos	68	244	3	4.8	v + o	25.5
A.A.	Post	2	Neg	12	22	2	24.7	v	9.1
D.D.	Pre	4	Pos	345	444	3	16.4	v	22.7
D.G.	Post	4	Pos	116	288	3	6.2	v	13.72
E.H.	Pre	2	Pos	57	13	3	12.6	v	12.32
F.J.	Post	3	Neg	8	4	3	9.4	v	11.64
J.R.	Post	2	Pos	6	3	3	2.6	v	12.04
K.U.	Pre	1	Pos	78	44	3	10.2	v	5.5
S.U.	Post	2	Pos	14	1	2	6.7	v	8.54
W.I.	Post	3	Pos	1	2	3	7.3	v	15.92

[a] According UICC criteria.
[b] Positive, ≥20 fmol/mg protein.
[c] Positive, ≥5%.
[d] o, osseous; v, visceral.

Table 3 Adjuvant systemic therapy (n = 388)

Therapy	No.	BSP ≥24 *n* (%)	BSP <24 *n* (%)
Endocrine therapy (Tamoxifen, Goserelin)	244	21 (72%)	223 (62%)
Chemotherapy (CMF, EC, FEC)[a]	114	8 (28%)	106 (30%)
Chemotherapy + endocrine therapy	19	0	19 (5%)
No adjuvant treatment	11	0	11 (3%)

[a] CMF, cyclophosphamide methotrexate, and 5-FU; EC, epirubicin, cyclophosphamide; FEC, 5-FU, epirubicin, cyclophosphamide.

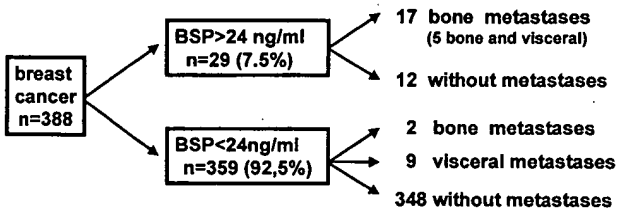

Fig. 1 Pattern of metastasis in patients with breast cancer, depending on preoperative BSP values (< or >24 ng/ml).

Statistical Analysis

The association of BSP values < and >24 ng/ml with established prognostic factors was analyzed by χ^2 tests for contingency tables. Survival methods were applied to bone metastasis-free survival (defined as survival without the development of bony metastases). The survival curve was calculated by the Kaplan-Meier method, based on the log-rank test according to Mantel and Breslow. A stepwise multivariate Cox regression analysis was performed to assess the independent prognostic value of serum BSP in comparison with other prognostic factors. The impact of each variable in the Cox regression model was tested by the Wald χ^2 test and described by the risk ratio (*i.e.*, the hazard ratio). All reported *P*s are two-sided. The statistical analysis was done with the aid of SAS (SAS Institute, Inc., Cary, NC) and Systat (Evanston, IL) software.

Results

Characteristics of the Breast Cancer Patients. The median age of the patients was 55 years (range, 24–82). The clinical and pathological characteristics of all 388 patients with breast cancer are shown in Table 1. One hundred and ninety-five women (50%) had a primary tumor <2 cm in diameter (T_1). In 156 patients (40%), the tumor had a diameter of between 2 and 5 cm, and 37 women (10%) had T_3 or T_4 tumors. There was no axillary involvement in 252 patients (65%), whereas 136 (35%) had histologically confirmed metastases in the axillary lymph nodes. Of 357 primary tumors examined, 226 (63%) were estrogen receptor positive; 228

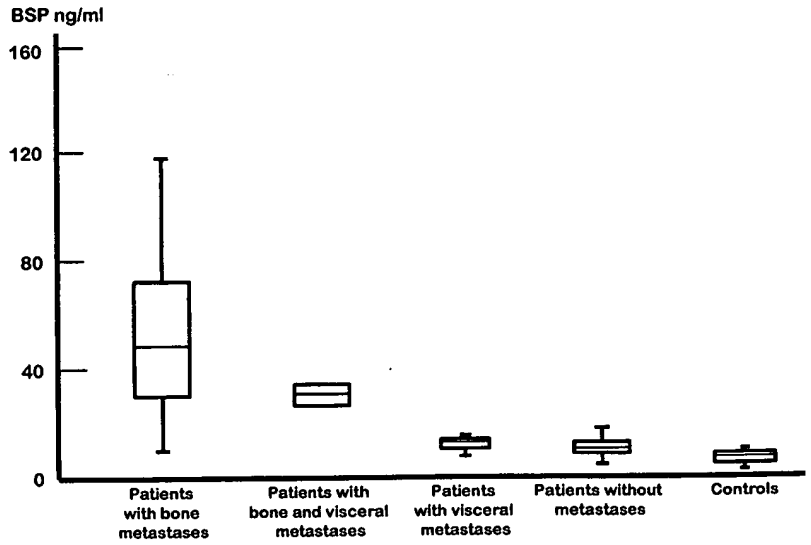

Fig. 2 Box and whiskers plot of BSP values in patients with bone metastases (n = 19), bone and visceral metastases (n = 5), visceral metastases (n = 14), without metastases (n = 360), and in controls (n = 30). In the plot, the *center horizontal line* marks the median of the sample. The *edges* (hinges), mark the first and third quantiles. The *whiskers* show the range of values that fall within 1.5 Hspreads of the hinges.

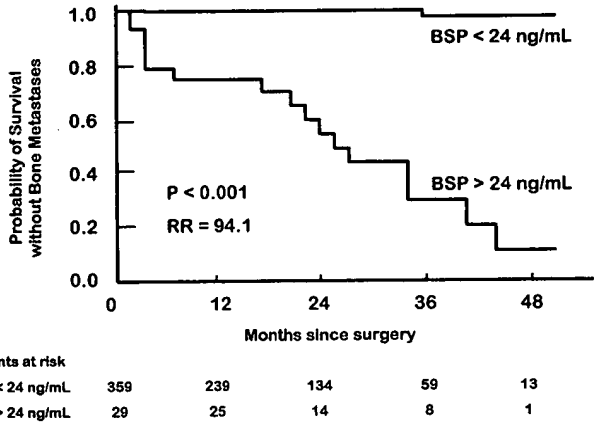

Fig. 3 Bone metastases-free survival in patients with primary breast cancer according to the serum BSP values.

Table 4 Results of univariate and multivariate analysis with regard to metastasis-free survival[a]

	P	RR[b]	95% CI[b]
Univariate analysis			
Bone sialoprotein (<24, ≥24)	<0.001	94.10	21.67–408.72
Tumor size[c] (T_1, T_2, T_3, T_4)	0.018	3.32	1.23–8.98
Nodal status[c] (N_0, N_{1-3}, N_{4-9}, N_{10+})	0.003	1.78	1.19–2.66
S-phase fraction (<5%, ≥5%)	0.127	2.12	0.81–5.59
Estrogen receptor[d] (positive, negative)	0.789	1.13	0.45–2.83
Progesterone receptor[d] (positive, negative)	0.578	1.29	0.52–3.22
Grading[c] (I+II, III)	0.723	1.18	0.46–3.02
Multivariate analysis			
Bone sialoprotein (<24, ≥24)	<0.001	93.96	21.65–408.30
Nodal status[c] (N_0, N_{1-3}, N_{4-9}, N_{10+})	0.083	4.434	2.96–5.91

[a] Cox regression stratified by adjuvant therapy.
[b] RR refers to the comparison of one category to the next. Tumor staging is according to UICC criteria. CI, confidence interval.
[c] Tumor staging according UICC criteria.
[d] Posistive, ≥20 fmol/mg protein.

of 358 tumors tested (64%) were progesterone receptor positive. A total of 127 patients (33%) were premenopausal; 261 (67%) were postmenopausal. With regard to grading, 247 of 377 tumors examined (66%) were assessed as grade I or II, and 130 (34%) were grade III. The S-phase fraction was <5% in 106 of 358 tumors (30%) and >5% in 252 cases (70%).

Results of Preoperative Serum BSP Determinations. The mean value for serum BSP for all patients with breast cancer was 14.1 ng/ml (range, 1.9–152.9). There were 29 patients (7.5%) with a value in excess of 24 ng/ml. The mean value for serum BSP in patients with benign breast disease was 8.8 ng/ml (range, 3.2–16.3).

Using the χ^2 test, a significant difference between patients with serum BSP values ≥24 *versus* <24 ng/ml with established prognostic factors was only seen for tumor size (P = 0.001; Table 1). Notably, the highest serum BSP values were seen in patients with T_1 and T_2 tumors (Table 2). There were no significant differences for nodal status (P = 0.251), estrogen-receptor content (P = 0.885), progesterone-receptor content (P = 0.831), tumor grading (P = 1.0), S-phase fraction (P = 0.243), or for menopausal status (P = 0.539).

Surgical and Adjuvant Systemic Treatment. Breast conserving therapy was possible in 261 of 388 patients (67%); mastectomy was performed in 127 patients (33%). Almost all patients (n = 377; 97%) received adjuvant systemic treatment (Table 3). Endocrine therapy was given to 72% (21 of 29) of the patients with a BSP value ≥24 ng/ml and to 62% (223 of 359) of the patients with a BSP value ≤24 ng/ml. Cytotoxic chemotherapy was used in 28% (8 of 29) of the patients with elevated BSP levels and in 30% (106 of 359) of those with normal BSP. The 19 patients (5%) who received a combination of chemotherapy and hormone therapy and the 11 women (3%) who received no systemic therapy all had normal BSP levels.

Follow-Up and Pattern of Metastasis. During the median observation period of 20 months, distant metastases were detected in 28 patients. Metastases to bone only were seen in 14 patients, 9 women developed purely visceral metastases (liver, lungs, and

central nervous system), and a combination of osseous and visceral metastases was observed in 5 patients (Fig. 1). Seventeen of the 19 women with osseous metastasis (alone or in combination) had preoperative serum BSP values in excess of 24 ng/ml, compared with none of the 9 women with purely visceral metastasis. The median values with respect to the various metastatic sites are shown in Fig. 2. The median serum BSP value was 48.3 ng/ml (range, 9.53–152.9) in patients with metastases to bone only, 30.6 ng/ml (range, 25.5–59.5) in patients with both osseous and visceral metastases, and 12.3 ng/ml (range 5.5–22.7) in patients with visceral metastases only. In patients without metastases, the median value was 9.7 ng/ml (range, 1.9–120), and in the controls, 8.7 ng/ml (range. 3.2–16.3). Twelve women with a preoperative BSP value of 24 ng/ml or more were free from metastases at the time of follow-up. Calculated sensitivity was 89.5%, and specificity was 96.7%; positive prediction was 58.6%, and negative prediction was 99.4%.

Prognostic Value of Serum BSP for Osseous Metastases. The Kaplan-Meier curve (Fig. 3) showed significant differences with regard to subsequent skeletal metastases between the two groups of patients with BSP values $<$ and $\geq$24 ng/ml. Thus, patients with elevated serum BSP values had a significantly higher risk of developing skeletal metastasis within 2 years after surgery.

Table 4 shows the results of the univariate and multivariate Cox regression analyses stratified according to adjuvant therapy. Serum BSP was investigated as an independent prognostic factor for subsequent bone metastasis. In the univariate analysis, BSP proved to be the best indicator for skeletal metastasis with a P of <0.001 and a RR of 94.10. This was followed by the size of the primary tumor ($P = 0.018$; RR, 3.32) and the nodal status ($P = 0.003$; RR, 1.78). In the multivariate analysis, only BSP remained as a prognostic factor after stepwise regression ($P < 0.001$; RR, 93.96). Calculations including patients with visceral metastasis only revealed no significant correlation for BSP as an independent prognostic factor ($P = 0.38$; data not shown).

Discussion

In our investigation of the prognostic value of serum BSP in patients with primary breast cancer, we were able to show that patients with BSP values in excess of two SDs of the normal value for postmenopausal patients (>24 ng/ml) were at an enormous risk of developing skeletal metastases in the first 2–3 years after the diagnosis of disease. This is in keeping with previous immunohistochemical studies indicating an unfavorable prognosis for women with breast cancer in which the primary tumor demonstrated positive immunoreactivity for BSP (14, 15, 20, 21). The significance of the present observations, however, lies in the use of a specific and sensitive RIA that is simple to use in clinical routine and functions without the subjective assessment of immunohistochemical staining. The method also offers the possibility of carrying out follow-up determinations.

BSP, a phosphorylated glycoprotein with a molecular weight of about M_r 70,000–80,000, accounts for ~10% of noncollagenous bone matrix proteins (8–10). BSP and its mRNA has been demonstrated in osteoclasts and osteoblasts but also in trophoblastic cells and in platelets. BSP contains an Arg-Gly-Asp (RGD) integrin recognition sequence. It improves the attachment of osteoclasts and osteoblasts, nucleates hydroxyapatite formation, and appears to enhance osteoclast-mediated bone resorption (22–33) The fact that microcalcifications in breast cancers consist of hydroxyapatite as well as calcium oxalate was the rationale for investigating BSP and other bone matrix proteins in primary tumors in breast cancer patients (34).

Ultimately, bone metastases are induced by the same mechanisms as all other metastases. There are, however, mutual interactions between tumor cells and the skeleton that determine organ selectivity. From *in vitro* studies by Mundy *et al.* (35) and Orr *et al.* (36), it is known that substances released from resorbing bone have a chemotactic effect on tumor cells. Furthermore, tumor cells that reach bone require adhesion molecules to become attached to the bone surface. BSP, with its integrin recognition RGD sequence, may play a part in the adhesion process. This has already been demonstrated for BSP-modulated adhesion of osteoclasts and osteoblasts (13, 29, 37, 38). It is possible that tumor cells are primed and coated with BSP in the primary tumor and are then able to adhere and bind to the bone surface. In cell attachment assays, Van der Pluijm *et al.* (39) have been able to show that the adhesion of breast cancer cell lines to bone surfaces could be successfully inhibited by application of BSP peptides.

To a large extent, the marked elevations in serum BSP seen in our study were probably attributable to paraneoplastic production by the primary tumor. This hypothesis is supported by the positive correlation between BSP and the size of the primary tumor (Table 1). However, circulating BSP may also be derived in part from normal or abnormal bone turnover. Seibel *et al.* (18) showed that serum BSP levels were elevated in patients with breast cancer and skeletal metastases, multiple myeloma, Paget's disease, and primary hyperparathyroidism. In our study, an increase in bone turnover could be caused, for example, by paraneoplastic production of PTHrP, as has been reported in breast cancer in a number of studies (6, 7, 40, 41). Further studies including repeated measurement of BSP after primary surgery are presently under way to confirm this hypothesis.

Despite the fact that osteotropic metastasis in breast cancer has long been recognized, there is still no reliable marker to predict an elevated risk of subsequent bone metastasis. Although it is known that well-differentiated breast cancers (grade I and II) are associated with bone metastasis more frequently than poorly differentiated tumors (grade III), and although the same is true of primary tumors with a high steroid receptor content, these factors have no predictive impact for skeletal metastasis (3–5). It is also known that tumors that are immunoreactive to PTHrP also preferentially metastasize to bone. The presently available serum tests for PTHrP are not sufficiently sensitive to replace immunohistochemical detection. Elevated PTHrP values in serum can only be reliably detected in patients with humoral hypercalcemia of malignancy (42).

The determination of serum BSP might provide a reliable marker for subsequent bone metastasis. However, it should be pointed out that in our group of 388 patients, skeletal metastasis in the further course of the disease would be expected in 80–100 cases. In contrast, there were only 29 women with BSP values in excess of 24 ng/ml, which corresponds to only about a third of the number expected to develop bone metastases. Of these 29 patients, 17 (60%) already had osseous metastases after a median follow-up of only 2 years. We therefore suspect that BSP measurements at the time of surgery are primarily predictive of early osseous metastasis, and there may be different pathways for early and late bone metastases. Renewed follow-up in 1–2 years will provide a more exact answer to this question.

There is no doubt that breast cancer patients with elevated serum BSP constitute a high-risk group that might benefit from early treatment with osteoprotective drugs. A promising group of such substances are the bisphosphonates. In a recently published study, we showed that breast cancer patients who received adjuvant therapy with the bisphosphonate clodronate (1600 mg/day p.o. for 2 years) developed fewer bone metastases and fewer visceral metastases than patients in a control group (43). A further placebo-controlled study in more than 1000 patients confirmed our findings, at least with regard to the prevention of bone metastases (44).

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